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Construction of the RapiDeg yeast strain

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An abbreviated version of this protocol was published in eLIFE in Jun 2017

ESCRTs function directly on the lysosome membrane to downregulate ubiquitinated lysosomal membrane proteins

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Detailed protocol

Protocol for "Construction of the RapiDeg yeast strain"

1. Plasmids
MLP479: pRS305-pTDH3-FRB-3xUb (see attached vector map)
MLP379:pFA6a-GFP-2xFKBP-TRP1 (see attached vector map)

2. Strains
SEY6210: MATα, ura3-52 his3Δ-200 leu2-3,112 trp1-901 lys2-801suc2Δ9(Robinson et al., 1988)
SEY6210.1: MATa, ura3-52 his3Δ-200 leu2-3,112 trp1-901 lys2-801suc2Δ9(Robinson et al., 1988)
LZY273: SEY6210.1;tor1-1 fpr1Δ::natMX
LZY562: SEY6210.1;tor1-1 fpr1Δ::natMX leu2–3,112::pRS305-PTDH3FRB-3xUb CAN1::CAN1-GFP-2xFKBP-TRP VPH1::VPH1-mCherry-kanMX6

3. Growth media
YPD media: Yeast extract, 1% (mv); Peptone, 2% (m/v); agar (for plate use only, 2%(m/v)); dextrose, 2%(m/v).
Yeast synthetic growth media: Yeast nitrogen base (with $(NH_4)_2SO_4$), 0.67% (m/v); supplemented with necessary amino acids; agar (for plate use only, 2% (m/v)); dextrose, 2%(m/v).

4. Generating and validating the RapiDeg strain
When necessary, gene deletion and tagging were made using standard yeast genetic replacement technique with longtine-based PCR cassettes (Longtine et al., 1998).
4.1 Construct the RapiDeg parent strain
(1) WT strains (SEY6210 or SEY6210.1) were made rapamycin-resistant by transforming a PCR fragment encoding the dominant *TOR1* mutant (S1972I) and selected for growth on YPD plates (supplemented with 1 μg/ml rapamycin). The tor1-1 mutant was further validated by rapamycin resistance and *TOR1* sequence.
(2) *FPR1* (FKBP-12) gene was knocked out to ensure pairing of FRB-3xUb to target proteins tagged with FKBP.

4.2 Cargo protein tagging
(1) Design the primers to C-terminally tag the protein of interest with GFP-2xFKBP, using MLF379 as the template;
(2) PCR amplify the C-terminal tagging fragment with homology to the C-terminus of the cargo protein. In the RapiDeg parent strain constructed in the step 4.1, perform standard genetic procedures to fuse the protein of interest with a C-terminal GFP-2xFKBP and culture the yeast on appropriate selection plate.

4.3 Cargo sorting assay
(1) The strain generated in the 4.2 will be further transformed with FRB-3xUb expression vector MLP479 and empty vector pRS305 as a negative control;
(2) Culture this RapiDeg strain (Cargo protein is tagged with C-terminal GFP-2xFKBP) co-expressing the FRB-3xUb in either YPD or appropriate minima medium. Grow the culture to mid-log phase (OD_{600}~0.6) and add rapamycin to 1μg/mL (final working concentration). Collect the cells for fluorescence microscopy and western blot analysis.

Related files

pFA6a-2xFKBP-GFP-TRP.pdf

pRS305-pTDH3-FRB-Ub.pdf

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Zhu, L. (2020). Construction of the RapiDeg yeast strain. Bio-protocol Preprint. bio-protocol.org/prep232.
2. Zhu, L., Jorgensen, J. R., Li, M., Chuang, Y. and Emr, S. D.(2017). ESCRTs function directly on the lysosome membrane to downregulate ubiquitinated lysosomal membrane proteins. eLIFE. DOI: 10.7554/eLife.26403